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Phytochemical analysis and antifungal potential of two *Launaea mucronata* (Forssk.) Muschl and *Launaea nudicaulis* (L.) Hook.fil. wildly growing in Anbar province, Iraq

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A R T I C L E I N F O

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A B S T R A C T

Ethnopharmacological relevance: Plant fungi are a serious problem in agriculture. Even though synthetic fungicides are an efficient control method, several negative side effects emerge from their extensive use, such as health problems, environmental pollution, and the emergence of resistant microorganisms. Thus, it is becoming more and more urgent to search for alternative control methods.

Aim of the study: The aim of our study was to analyze phytochemical composition and antifungal potential of ***Launaea mucronata*** (Forssk.) Muschl. and ***Launaea nudicaulis*** (L.) Hook. fil**.** wildly growing in Anbar province, Iraq. In addition, molecular analysis was used to identify the plants species and molecular docking analysis was investigated between the major phytochemicals present in these plants and three selected fungal proteins, in order to assess the antifungal activity of the selected biochemicals against these proteins.

Materials and methods: Molecular analysis was performed using ITS sequencing protocol. The phytochemical analysis was done using GC-MS technique, while molecular docking analysis was performed by FRED application between selected compounds from each plant and three enzymes: 17β-hydroxysteroid dehydrogenase, endochitinase, and 14-α-demethylase. Finally, the antifungal activity was assessed by measuring inhibition percentage of *Fusarium solani* and *Macrophomina phaseolina* growth treated with ethanomethanolic extract of each plant.

Results: Molecular analysis identified the selected plants as *L. mucronata* and *L. nudicaulis,* with an ITS region of 600 bp. Phytochemical analysis of *Launaea* spp. reported the presence of 35 compounds in each ethanomethanolic extract, belonging to different classes. *L. mucronata* was mainly formed of lupeol (9.33%), whereas *L. nudicaulis* extract was dominated by the heterocyclic compound 4-(3-methoxyphenoxy)-1,2,5-oxadiazol-3-amine (20.2%). Furthermore, molecular docking analysis showed that 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl from *L. mucronata* and gulonic acid ϒ-lactone from *L. nudicaulis* possessed the highest affinity score to 17-β-hydroxysteroid dehydrogenase (−4.584 and −7.811 kcal/mol, respectively). Sucrose from L. *mucronata* and glutaric acid, di(3,4-difluorobenzyl) ester from *L. nudicaulis* gave the highest affinity to endochitinase (−7.979 and - 8.446 kcal/mol, respectively). In addition, sterol 14-α-demethylase was affinitive to sucrose from *L. mucronata* and glutaric acid, di(3,4-difluorobenzyl) ester from *L. nudicaulis via* energetic score of −10.002 and −9.582 kcal/mol, respectively. Both extracts exhibited antifungal activity against *F. solani* and *M. phaseolina* in a dose-dependent manner.

Conclusions: This study confirms the antifungal potential of both *Launaea* spp. explained by the presence of phytochemicals with antimicrobial properties. These compounds have potential to be used as new drugs to treat

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infectious diseases caused by pathogens. Consequently, plants from Launaea genus could be a raw material for many studies such as therapeutic, taxonomical, drug modelling, and antifungal agent.

Abbreviations

ASL	Above Sea Level
DMSO	Dimethyl Sulfoxide;
DNA	Deoxyribonucleic acid
E	East
GC-MS	Gas Chromatography - Mass Spectroscopy
ITS	Internal Transcribed Spacer
LM	*Launaea mucronata*
LN	*Launaea nudicaulis*
M	Marker
MSTFA	N-methyl-N-(trimethylsilyl) trifluoroacetamide;
N	North
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
TBE	Tris/Borate/EDTA
ZR	Zymo Research

1. Introduction

Launaea is a genus of Asteraceae family including more than 40 species of medicinal importance, inhabiting mainly arid and semi-arid regions (Noumi et al., 2010). Locals in Saharan regions called some of Launaea plants "Marar" (from the Arabic word "Murr"), which means "bitter" due to the bitterness that characterizes camel's milk after they graze on these plants (Mandaville, 2019). Traditionally, *Launaea* spp. have been used for the treatment of several diseases like fever, itches, liver, lungs and stomach illnesses, ulcers, as well as to heal infected cuts (Cheriti et al., 2012). Extracts of *Launaea* spp. have exhibited several activities, such as antibacterial (Benmeddour et al., 2015; Moussaoui et al., 2010; Parekh and Chanda, 2006), antifungal (Moussaoui et al., 2010), antiparasitic (Bremer Christensen et al., 2015), nephroprotective (Khan et al., 2012b), antiurolithiatic (Makasana et al., 2014), antioxidant (Khan et al., 2012a), and allelopathic activities (Khan et al., 2011). *L. mucronata* and *L. nudicaulis* are two plants belonging to Launaea genus exhibiting potential as anticancer (Abouzied et al., 2021; Elsharkawy, 2017; El-Sharkawy and Mahmoud, 2016), antioxidant (Abouzied et al., 2021; Elsharkawy, 2017), antibacterial (Al-Mahrezi et al., 2011), and antidiabetic agents (El-Newary et al., 2021). These activities are attributed to secondary metabolites synthetized by Launaea species, including terpenoids, sesquiterpenoids, and flavonoids (Cheriti et al., 2012).

Plant pathogens have undeniable destructive role and they are constantly developing innovative mechanisms to avoid control methods. Thus, searching for alternative ecofriendly control methods with low cost is becoming necessary. Even though chemical-based practices are fast and efficient, considerable attention should be given to the emerging resistant microorganisms as well as growing concerns of environmental pollution that cannot be overlooked (Pandit et al., 2022). Indeed, the development of drug-resistance in microorganism is an increasing global problem. For fungal infection, resistance rates vary by geographic region, types, and available therapeutic choices. However, spread of drug-resistant fungal is universal. Usually, innate and acquired resistance mechanisms of fungal pathogens are related to a decrease in efficient drug concentration, alteration in drug target, and redirecting antifungal toxicity *via* metabolic shifting (Geddes-McAlister and Shapiro, 2019). Fungi tolerate or resist drugs using genetic factors, including stable inheritable mutation (point mutations), and/or epigenetic factors, involving DNA methylation, histones deacetylation, chromatin remodeling, and RNA interference in mediating drug-resistant fungi without altering their DNA sequence. Recent reports indicated that the tolerance of fungi to certain chemically synthesized medications is due to slow grow of some cells undergoing treatment by these chemicals (Gow et al., 2022). Thus, there is an urgent need to seek new compounds from natural sources, having a different mode of action that would enable them to bypass the resistance mechanism developed by fungi. Due to their wild nature, many plant species growing in desert regions, such as Launaea, have developed defense mechanisms that help them survive in hostile growth conditions (Neama and Almehemdi, 2018). One of the most prominent defense mechanisms of plants is the increased accumulation of certain active components or secondary metabolites that have antifungal, antioxidant, or antimicrobial properties, improving opportunity of wild species to survive and have a successful life cycle (Abou-Zeid et al., 2008; El-Sharkawy et al., 2017).

Therefore, this study aims to assess molecular and phytochemical properties of *L. mucronata* and *L. nudicaulis* growing in Anbar province. Antifungal activity of ethanomethanolic extract of the two species is also evaluated against *Fusarium solani* and *Macrophomina phaseolina* under *in vitro* conditions.

2. Material and methods

2.1. Collection of plant material

A fresh sample of wild *L. mucronata* and *L. nudicaulis* was collected at the blossoming stage in 15 December 2021 (Fig. 1) from bare land in the campus of University of Anbar, Anbar, Iraq, 33°24'24.102" N, 43°15'44.8848" E and 56 m ASL (above sea level). Whole plants were extirpated and transferred directly to the lab in polyethylene bags. The morphological identification and authentication of the collected plants were made by Prof. Dr. Mohammed Othman Mosa, Center of Desert Studies, University of Anbar, Iraq. Plant materials of *Launaea* spp. were deposited in the National Herbarium of Iraq with vouchers 25726 and 6304 for *L. mucronata* and *L. nudicaulis*, respectively. The two species were checked thoroughly with the data on "the Plant List" (www.theplantlist.org). The accepted name record of *L. mucronata* is 2D6FEC52-6E87-4CED-94E2-253A6C2BF333, and of *L. nudicaulis* is 8878DC1A-A9A3-4E3C-9E23-D354EFA8FD82.

2.2. Molecular analysis

2.2.1. DNA extraction, quantification, and qualification

Total genomic DNA was extracted from 100 mg of fresh leaves of each species with aid of ZR Plant/Seed DNA MiniPrep™ (Zymo/USA). The extraction procedure was followed as the supplier instructed. Then, the extracted DNA was quantified and qualified using Nanodrop read between 1.8 and 2, and finally adjusted to a work concentration of 50 ng/μL.

2.2.2. Polymerase chain reaction (PCR)

The PCR amplification was conducted in a total volume of 25 μL, containing 1.5 μL DNA, 5 mL Taq PCR PreMix (Intron, Korea), and 1 μL of each primer (10 pmol). Distilled water was added to reach a total volume of 25 μL. Two specific primers (Integrated DNA Technologies Company-IDT/Canada) were used to amplify the ITS region; ITS1 as forward (F: 5′- TCCGTAGGTGAACCTGCGG -3′) and ITS4 as a reverse (R: 5′- TCCTCCGCTTATTGATATGC -3′).

PCR thermal profile was as follow: Denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 45 s, 52 °C for 1 min, and 72 °C for 1

min, with final incubation at 72 °C for 7 min using a thermal cycler (Gene Amp, PCR system 9700; Applied Biosystem). The amplification products were separated on 1.5% agarose gel electrophoresis and visualized by ultraviolet light (302 nm) after red stain staining (Intron, Korea).

2.2.3. Agarose gel electrophoresis

Agarose gel was made in 1.5% condensation by melting 1.5 g of agarose in a 100 ml of previously prepared 1x TBE buffer according to Sambrook and Russell (2001). Ten microliters of each PCR sample was carefully loaded into each well. Then, electrophoresis was performed at 5 V/cm gel length for 2 h until the dye front reaches the end of the gel. Nesxt, electrophoresis gel was stained in a staining solution pool (3 μL Red safe Nucleic acid and 500 mL of distilled water), visualized on a 336 nm UV light, and photographed.

2.2.4. ITS sequencing protocol

Gel extraction DNA was accomplished as described by Vogelstein and Gillespie (1979). Sanger sequencing method was adopted to sequence the gel extracted DNA in the National Instrumentation Center for Environmental Management- NICEM (http://nicem.snu.ac.kr/main/?en_skin=index.html), Biotechnology lab using 3730XL Genetic Analyzer machine (Applied Biosystems, USA). Homology comparisons of the query ITS sequences were conducted using Basic Local Alignment Search Tool (BLAST) software at the National Center Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov).

2.3. Preparation of ethanomethanolic extracts

Aerial parts of the collected plants were separated, washed, and left to completely dry under room temperature (25 °C ± 2) for one week. Home grinder was used to grind the plant material into a fine powder. From each species, 50 g of ground plant material were macerated in 2 L mixed solvents of 99.9% ethanol +97.9% methanol (1:1) overnight. Subsequently, ethanomethanolic extract was ultrasonicated in water bath for 50 min (39 °C). Then, crude extracts were filtered with Whatman filter paper grade 2 (Whatman, Kent, UK) under vacuum pump using Buchner funnel. Filtrates were concentrated with aid of rotary evaporator, and weighed. The total weight of the ethanomethanolic extracts of *L. mucronata* and *L. nudicaulis* were 3.5 g and 2.9 g, respectively.

2.4. Phytochemical analysis

The phytochemical profile of the ethanomethanolic extracts was studied using gas chromatography with electron impact mass spectrometry analysis (GC-MS). We used a GC-MS-QP2010 plus instrument (Shimadzu, Kyoto, Japan) equipped with autoinjector and 5 ms capillary column of 30 × 0.25 mm dimension with 0.25 μm film thickness. Helium served as the carrier gas at 1.15 mL/min flow rate. Mass spectroscopic analysis was done with 70eV ionization system. The primary temperature was established at 80 °C for 2 min and gradually elevated at a rate of 10 °C per min up to 280 °C for 5 min. The sample was injected according to split mode at 250 °C. According to retention time and mass spectra, the extracted bioactive compounds were identified by comparing their mass spectra with two reference mass spectral databases: National Institute of Standards and Technology (NIST14) and Wiley 10th/NIST 2014 mass spectral library (W10N14). Derivatization step was adopted prior to GC-MS analysis, using N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) reagent and reference standards (Sigma Aldrich, St. Louis, MO, USA), to estimate sugars and other polar compounds present in our extracts (Rohloff, 2015). A calibration curves were constructed by plotting the peak areas against five different concentrations (μg/mL) of each standard. Furthermore, five replicates were used for each calibration level.

2.5. Molecular docking study

All chemical structure conformations were generated using the OpenEye scientific software package (Hawkins et al., 2007). Geometry optimization procedures were then performed by force field mechanics MMFF94 with the option of no ionization change and chirality determination from the 3D structure using the OMEGA application. The 3D crystal structure of 17β-hydroxysteroid dehydrogenase (pdb: 3IS3), endochitinase (pdb: 4TXE) and sterol 14-alpha demethylase (CYP51B) (pdb: 5FRB) were obtained from the Protein Data Bank. The Protein Preparation Wizard tool was used to remove water, ions, and attached ligands, with optimization and minimization limited by the MMFF94 force field mechanism. Pairing and interactive association evaluation were performed using the FRED application in the center of each active page in the defined grid, with the grid box size adjusted to 50 × 50 × 50 Å and the partial atomic charge to 0.27. During coupling, the ligands were made flexible while the receiver remained rigid. For ligand connectivity, an additional precision model with flexible ligand sampling parameters was selected. The 2D structures of the compounds were

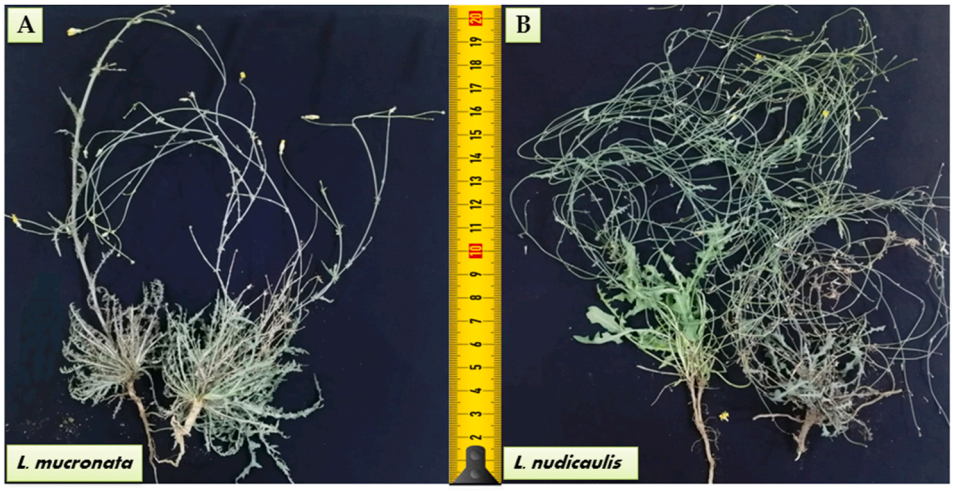


Fig. 1. Morphology of L. *mucronata* (A) and *L. nudicaulis* (B).

downloaded from the online PubChem database (https://pubchem.ncbi.nlm.nih.gov/). All results have been aggregated and exported as Excel files for further analysis and interpretation.

2.6. Antifungal study

2.6.1. Field survey

A field survey was conducted to collect soybean plants with wilt symptoms from three governorates, Baghdad (Abu-Ghraib), Anbar (Khalidiya and Ramadi), and Salah Al-Din (Tikrit) during May and June of the year 2021. Plants that showed symptoms of stem base discoloration with a dark brown or blackish color, necrosis, or wilting were collected and placed in separate polyethylene bag to be transferred to the lab for further use.

2.6.2. Isolation of fungi

The collected plants were washed with tap water to remove soil residue, then after superficial sterilization, smaller cuts (0.5–1 cm) were sterilized with NaOCl (5%) for 2–3 min. Sterile water was used to wash the previously sterilized cuts, then left to dry on sterile filter paper. In order to allow fungal growth, the cuts were cultured on PDA (Potato Dextrose Agar) supplemented with antibiotic (Amoxicillin) and incubated at 25 ± 2 °C for three days. Fungal hyphae grown on the medium were transferred by a sterile needle to a new plate containing sterile PDA and incubated at 25 ± 2 °C for seven days for diagnostic purposes.

2.6.3. Identification of fungi

The isolated fungi were identified according to their morphological characteristics concerning *M. phaseolina* (Veverka et al., 2008). *F. solani* was identified based on the characteristics of the fungal culture and the microscopic characteristics mentioned in Chehri et al. (2015).

2.6.4. In vitro antifungal investigation

To investigate the inhibitory effect of ethanomethanolic extracts on the two selected plant pathogenic fungi, the concentrated residues were first dissolved in 2.5% dimethyl sulfoxide (DMSO; Sigma-Aldrich Co., MO, USA) and diluted into three concentrations using the same solvent (0.01, 0.001, and 0.0001 mg/L) plus DMSO (2.5%). A volume of 5 mL from these concentrations were added to PDA medium before solidification. The medium was then poured into Petri dishes of 9 cm diameter. The negative and positive controls contained DMSO (25 μL) and Azoxystrobin (12 μg), respectively. After solidification, a disk of 0.5 cm diameter of 5 days old fungi was deposited on the PDA medium. Petri dishes were incubated at a temperature of 25 ± 2 °C for 10 days. All experimental bioassays were performed in triplicate and the inhibitory effect of plant extracts on radial mycelial growth (IR) was determined by measuring the average diameter of colonies fungal growth according to the following equation (Pinto et al., 1998):

$$IR\ (\%) = Dc - Dt\ /\ Dc$$

with Dc: the average diameter of fungal growth of the negative control (DMSO), Dt: the average diameter of fungal growth treated with the plant extracts or the positive control.

3. Results and discussion

3.1. Molecular analysis of Launaea spp.

DNA barcoding is a valuable technique used frequently to distinguish between closely related or even recently evolved species, especially when morphological characterization is challenging. The used pair of primers (ITS1-ITS4) successfully amplified the targeted ITS region in both plant genomes with a molecular size of 600 bp (Fig. 2). Sequence of PCR amplified products were analyzed using Basic Local Alignment Search Tool (BLAST) software at the National Center Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov) for related references species. The results indicated that the two species were *L. mucronata* and *L. nudicaulis* deposited in GenBank under the accession numbers OP106590 and OP106591, respectively.

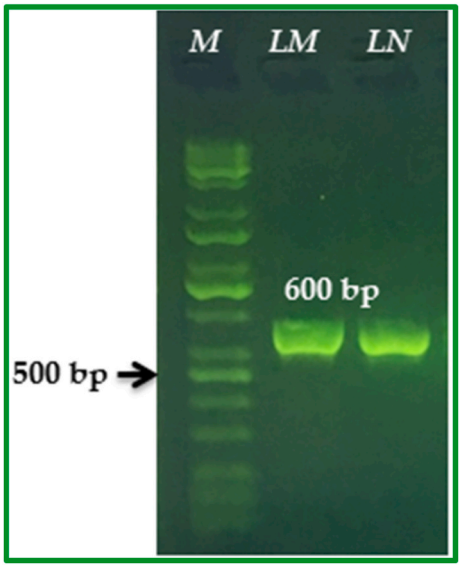


Fig. 2. PCR amplification products of ITS region of *L. mucronata* and *L. nudicaulis*.

M: Marker; LM: *L. mucronata;* LN: *L. nudicaulis*.

The study of the developmental phases is necessary for the analysis of the spatio-temporal changes of the molecular markers during the growth cycle, in order to analyze their evolution and the variations in some features depending on various other parameters (Wang et al., 2019). Different factors may affect the phytochemical composition, thus the potential of certain plant species. Research on several plants have shown that the genes involved in the secondary metabolite pathways are often in gene clusters that can evolve new functions by wide range of genetic and/or epigenetic alterations, such as duplication and reorganization (Alfalahi et al., 2022). In general, biosynthesis of secondary metabolites is a multistep enzymatic process through which final products will be produced. Considering the genetic origin of multi-step biosynthetic pathways of natural product, the sequential reductase steps did not originate from simple gene manipulation like duplication and differentiation, as might have been anticipated based on the similarity of substrates utilized and mechanisms of these reactions. The pattern of metabolite accumulation was compared between genotypes and at different times to understand the model and difference. Furthermore, the essential oil is versatile regulatory dominance likely transcriptional markers (Mishra et al., 2021).

Many techniques were developed and were efficient to classify many plant taxa. These techniques conceptualize how given taxa vary in their transcriptomics, proteomics, and metabolomics (Alami et al., 2022). Some plant taxa are different due to their molecular difference and chemical biosynthesis called chemotypes (Dawood et al., 2020).

3.2. Phytochemical composition of Launaea spp.

In this study, the complete phytochemical profile of *L. mucronata* and *L. nudicaulis* extracts was obtained. Thirty-five compounds were reported in each plant, belonging to different classes. The ethanomethanolic extract of *L. mucronata* was dominated by the terpenoid lupeol (9.33%), followed by 1-heneicosanol (9.06%), and methyl 2-hydroxy-octadeca-9,12,15-trienoate (6.55%) (Table 1).

The ethanomethanolic extract of *L. nudicaulis* was dominated by the heterocyclic compound 4-(3-methoxyphenoxy)-1,2,5-oxadiazol-3-

Table 1
Phytochemical constituents identified in ethanomethanolic extract of *L. mucronata* aerial parts using gas chromatography-mass spectrometry (GC-MS).

No.	Compound name	RT (min)	Area %	MW (g mol^{-1})	Formula	Classification of compound
1	1-methyl-2-pyrrolidinemethanol	3.94	1.25	115.1	$C_6H_{13}NO$	N-alkylpyrrolidine
2	Ethyl(dimethyl) ethoxysilane	4.43	1.91	132.2	$C_6H_{16}OSi$	Trialkylheterosilane
3	1-(2-Chloroethyl) pyrrolidine	4.95	1.83	170	$C_6H_{12}ClN$	Haloalkyl substituted pyrrolidine
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.78	2.09	144.1	$C_6H_8O_4$	Dihydropyranone
5	N,N,N'-Trimethyl-1,4-benzenediamine	9.29	2.02	150.22	$C_9H_{14}N_2$	Phenylalkylamine
6	Sucrose	11.50	6.33	342.3	$C_{12}H_{22}O_{11}$	Disaccharide
7	1[5'-(hydroxymethyl)furfuryl]pyrrolidine	12.01	2.72	181.2	$C_{10}H_{15}NO$	Aralkylamine
8	N-Cyclopentyl-N-ethylacetamide	12.24	1.34	155.2	$C_9H_{17}NO$	Tertiary carboxylic acid amide
9	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	12.51	1.07	180.2	$C_{11}H_{16}O$	Benzofuran derivative
10	Oxazole, 5-hexyl-2,4-dimethyl-	15.25	1.17	181.2	$C_{11}H_{19}NO$	2,4,5-trisubstituted oxazole
11	5-Ethylcyclopent-1-ene-1-carboxylic acid	15.63	1.64	140.1	$C_8H_{12}O_2$	Organic acid
12	6-Octen-1-ol, 3,7-dimethyl-, formate	16.44	4.79	184.2	$C_{11}H_{20}O_2$	Monoterpenoid
13	l-Inositol	16.74	1.49	180.1	$C_6H_{12}O_6$	Sugar alcohol
14	3,7,11,15-Tetramethyl-2-hexadecen-1-OL	16.93	1.35	296.5	$C_{20}H_{40}O$	Diterpene
15	D-chiro-Inositol, 3-O-(2-amino-4-((carboxyiminomethyl) amino)-2,3,4,6-tetradeoxy-.alpha.-D-arabino-hexopyranosyl)-	17.09	1.55	379.3	$C_{14}H_{25}N_3O_9$	Aminoglycoside antibiotic
16	Hexadecanoic acid, methyl ester	17.47	1.85	270.5	$C_{17}H_{34}O_2$	Fatty acid methyl ester
17	n-Hexadecanoic acid	17.95	4.72	256.42	$C_{16}H_{32}O_2$	Fatty acid
18	Cis-1-(4-methylphenyl)-2-(2-furyl) cyclopropane	18.35	2.03	212.29	$C_{15}H_{16}O$	Phenylfuran
19	11,14,17-Eicosatrienoic acid	19.54	2.21	306.5	$C_{20}H_{34}O_2$	Long-chain fatty acid
20	Phytol	19.67	6.18	296.5	$C_{20}H_{40}O$	Diterpene
21	Methyl 2-hydroxy-octadeca-9,12,15-trienoate	20.00	6.55	308.5	$C_{19}H_{32}O_3$	Polyenoic fatty acid
22	i-Propyl 9,12,15-octadecatrienoate	20.28	2.82	320.5	$C_{21}H_{36}O_2$	Ester of isopropyl alcohol and linoleic acid
23	Chrysene, 1,2,3,4,4a,7,8,9,10,11,12,12a-dodecahydro-	22.85	1.75	240.3	$C_{18}H_{24}$	Phenanthrene
24	Naphtho[2,1-b:7,8-b']difuran, 1,2,9,10-tetrahydro-2,9-dimethyl-	23.45	1.89	240	$C_{14}H_8O4$	Naphthofurans
25	Silane, [[(3.beta.,20S)-pregn-5-ene-3,20-diyl]bis(oxy)]bis[trimethyl	23.77	1.27	462.8	$C_{27}H_{50}O_2Si_2$	Pregnane steroid
26	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester	23.95	1.25	330.5	$C_{19}H_{38}O_4$	Monoacylglyceride
27	Tricyclo[6.6.0.0(3,6)]tetradeca-1(8),4,11-triene	25.48	1.59	186	$C_{14}H_{18}$	Polycyclic hydrocarbon
28	2H-Pyran, 2-(2-heptadecynyloxy)tetrahydro-	25.75	3.8	336.6	$C_{22}H_{40}O_2$	Oxane
29	Stigmasterol	26.19	2.37	412.7	$C_{29}H_{48}O$	Sesquiterpenoids
30	**1-Heneicosanol**	**27.81**	**9.06**	**312.6**	**$C_{21}H_{44}O$**	**Primary fatty alcohol**
31	4-(3-Methoxyphenoxy)-1,2,5-oxadiazol-3-amine	28.14	1.22	207.1	$C_9H_9N_3O_3$	Heterocyclic compound
32	beta-Amyrin	28.58	3.93	426.7	$C_{30}H_{50}O$	Pentacyclic triterpenoid
33	Hopa-22(29)-ene-3alpha-ol	28.84	1.44	426.7	$C_{30}H_{50}O$	Terpenoid
34	**Lupeol**	**30.00**	**9.33**	**426.7**	**$C_{30}H_{50}O$**	**Triterpene**
35	Vitamin E	31.23	2.2	430.7	$C_{29}H_{50}O_2$	Chromanol derivative

amine (20.2%), followed by the phytosterol β-sitosterol (11.93%), and the fatty alcohol 1-heptacosanol (8.83%) (Table 2).

In our study, *L. mucronata* and *L. nudicaulis* extracts were composed of several classes of compounds. *L. mucronata* extract was dominated by terpenes and terpenoids. Another study showed the presence of triterpene compounds in the chloroform fraction of *L. mucronata* methanol extract, along with coumarins (El-Sharkawy and Mahmoud, 2016). In addition, Elsharkawy (2017) found that *L. mucronata* methanolic extract was also formed of apigenin, quercetin, rutin, isorhamnetin-3-O-β-glucoside, quercetin-3-O-β-glucoside, apigenin-7-O-β-diglucoside, and kaempferol-3-o-rutinoside. On another hand, *L. nudicaulis* extract was formed mainly of fatty acids and their derivatives (24.86%) and heterocyclic compounds (24.29%). Similarly, El-Newary et al. (2021) reported that the ethanolic extract of *L. nudicaulis* was dominated by fatty acids, along with acyl glycerol, phenolics, terpenoids, and flavonoids. On another hand, methanol extract was composed of flavonoids, alkaloids, glycosides, sterols, and saponins (El-Sharkawy et al., 2017). Moreover, β-sitosterol found in our extract was also reported in the methanolic extract of *L. nudicaulis* from Pakistan, in addition to four new sphingolipids (Riaz et al., 2012). In another study, the ethyl acetate soluble fraction *L. nudicaulis* methanolic extract contained four compounds including a quinic acid derivative, a pentahydroxy acetylene analog, a flavone glycoside, and a sesquiterpene lactone (Saleem et al., 2012). Moreover, two ursene-type triterpenes (nudicauline A and nudicauline B) were identified in the ethyl-acetate soluble fraction of methanolic extract (Ahmed et al., 2006). These differences in composition found between our species could be due to the unique biosynthetic system for each species based on the genetic material (Elshamy et al., 2019).

The composition of plant extracts largely depends on the extraction technique. El-Sharkawy et al. (2017) found differences in chemical composition of *L. nudicaulis* methanol and chloroform extracts; both extracts were formed of alkaloids, flavonoids, and saponins, while only methanol extract contained sterols and glycosides. The solvent used influences the nature of compounds extracted since phytochemicals have different solubility and polarity to each solvent (de Boer et al., 2005; Parekh and Chanda, 2006). Indeed, an organic solvent, like methanol and ethanol used in our study, allows a higher solubility of active ingredients during extraction (de Boer et al., 2005).

3.3. Molecular docking study

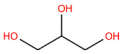
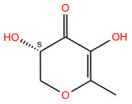
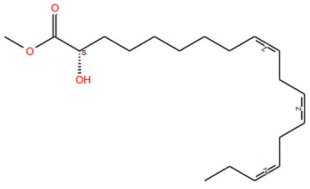
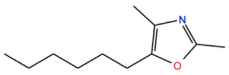
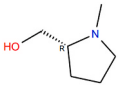
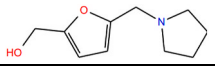
In this study, we have conducted a molecular docking between all the phytochemicals detected *via* GC/MS in the extracts of *L. mucronata* and *L. nudicaulis* and each of the three enzymes; 17β-hydroxysteroid dehydrogenase, endochitinase, and 14-α-demethylase. However, the five specific active constituents from each plant extract that showed the strongest binding affinity and can trigger the stimulation of the selected enzymes involved in biochemical processes were subjected to further investigation and discussion.

Several compounds from *L. mucronata* extract found to have strong binding affinity to specific amino acids forming the selected enzymes. The compound 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl had the strongest affinity to 17β-hydroxysteroid dehydrogenase (−4.584 kcal/mol) (Table 3). This result was due the highest affinity of this compound to the enzyme amino acid arginine 28 (ARG28) and its

Table 2
Phytochemical constituents identified in ethanomethanolic extract of *L. nudicaulis* aerial parts using gas chromatography-mass spectrometry (GC-MS).

No.	Compound name	RT (min)	Area %	MW (g mol^{-1})	Formula	Classification of compound
1	Silane, (2-ethoxyethoxy)trimethyl-	4.41	2.50	162.3	$C_7H_{18}O_2Si$	Trialkylheterosilanes
2	1-methyl-2-pyrrolidinemethanol	4.86	1.20	115.17	$C_6H_{13}NO$	N-alkylpyrrolidine
3	1-Butanamine, 2-methyl-N-(2-methylbutylidene)-	5.04	2.1	155.28	$C_{10}H_{21}N$	Butyl alcohol
4	2-Methyl-1-pentanol	5.30	1.30	102.17	$C_6H_{14}O$	Primary alcohol
5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	6.81	1.69	144.12	$C_6H_8O_4$	Flavonoid fraction
6	Azetidine, 1,1'-methylenebis[2-methyl-	10.24	1.69	154.25	$C_9H_{18}N$	Azetidine
7	Acetone, ethyl methyl acetal	11.27	1.70	118.18	$C_6H_{14}O_2$	Heterocyclic Organic Compound
8	1[5'-(hydroxymethyl)furfuryl]pyrrolidine	12.01	3.52	181.23	$C_{10}H_{15}NO_2$	Aralkylamine
9	Z-8-Methyl-9-tetradecenoic acid	12.25	1	240.38	$C_{15}H_{28}O_2$	Fatty acid methyl ester
10	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	12.53	1.22	180.24	$C_{11}H_{16}O_2$	Terpene
11	Glutaric acid, di(3,4-difluorobenzyl) ester	12.93	0.96	384.32	$C_{19}H_{16}F_4O_4$	Organofluorine compound
12	Citric acid	13.32	1.18	192.12	$C_6H_8O_7$	Tricarboxylic acid
13	cis-Decahydro-1-methylquinoline	15.27	1.15	153.26	$C_{10}H_{19}N$	Decahydroquinoline
14	1-Trimethylsilyl-3-(dimethyl-n-pentylsilyl)propane	16.16	1.07	244.6	$C_{13}H_{32}Si_2$	Alkyltrimethoxysilane
15	Gulonic acid gamma-lactone	16.45	5.48	178.14	$C_6H_{10}O_6$	Gamma butyrolactones
16	l-Inositol	16.74	1.68	180.16	$C_6H_{12}O_6$	Carbohydrate
17	E−10-Methyl-11-tetradecen-1-ol propionate	16.97	1.28	282.461	$C_{18}H_{34}O_2$	Alcoholic compound
18	Hexadecanoic acid, methyl ester	17.48	2.78	270.5	$C_{17}H_{34}O_2$	Fatty acid ester
19	n-Hexadecanoic acid	17.96	4.61	256.42	$C_{16}H_{32}O_2$	Saturated Fatty acid
20	6-Octadecenoic acid, methyl ester	19.62	7.97	296.5	$C_{19}H_{36}O_2$	Fatty acid ester
21	Methyl stearate	19.85	1.04	298.5	$C_{19}H_{38}O_2$	Fatty acid ester
22	Methyl 8,11,14-heptadecatrienoate	20.01	5.42	278.4	$C_{18}H_{30}O_2$	Fatty acid ester
23	Oleic Acid	20.27	2.04	282.5	$C_{18}H_{34}O_2$	Unsaturated Fatty acid
24	6-Nonenal, 3,7-dimethyl-	22.26	1.98	168.27	$C_{11}H_{20}O$	Aldehyde
25	Undecanal, 2-methyl-	23.41	1.35	184.32	$C_{12}H_{24}O$	Aldehyde
26	Stigmasterol	23.60	4.95	412.7	$C_{29}H_{48}O$	Sesquiterpenoids
27	**β-Sitosterol**	**25.67**	**11.93**	**414.7**	**$C_{29}H_{50}O$**	**Phytosterol**
28	**4-(3-Methoxyphenoxy)-1,2,5-oxadiazol-3-amine**	**26.07**	**21.29**	**207.19**	**$C_9H_9N_3O_3$**	**Heterocyclic compound**
29	1H-Inden-1-one, 2,3-dihydro-3,3,5,7-tetramethyl-	26.42	4.16	188.26	$C_{13}H_{16}O$	Polycyclic hydrocarbon
30	1H-Indene, 5-butyl-6-hexyloctahydro-	26.69	2.79	264.5	$C_{19}H_{36}$	Polycyclic hydrocarbon
31	2-Myristynoyl-glycinamide	27.06	2.23	280.41	$C_{16}H_{28}N_2O_2$	Amino compound
32	Cyclobarbital	27.49	2.36	236.27	$C_{12}H_{16}N_2O_3$	Barbiturates
33	1-Heptacosanol	27.86	8.83	396.7	$C_{27}H_{56}O$	Fatty alcohol
34	Stigmasta-3,5-dien-7-one	28.21	1.37	410.7	$C_{29}H_{46}O$	Steroid

Table 3
Molecular docking of phytochemical constituents identified in ethanomethanolic extract of *L. mucronata* aerial parts towards 17β-hydroxysteroid dehydrogenase (pdb: 3IS3).

No.	Compound name	Compound 2D structure	Binding score (Kcal/mol)
	Reference ligand (Glycerol)		−6.176
1	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl		−4.584
2	Methyl 2-hydroxy-octadeca-9,12,15-trienoate		- 3.824
3	Oxazole, 5-hexyl-2,4-dimethyl		−2.741
4	1-methyl-2-pyrrolidinemethanol		−2.714
5	1[5'-(hydroxymethyl)furfur		−2.709

hydrophobicity to isoleucine 30 (ILE30) (Fig. 3).

On another hand, sucrose from *L. mucronata* extract exhibited the highest affinity to endochitinase (- 7.979 kcal/mol), which was near to the reference ligand 38F (−8.23 kcal/mol), whereas the least affinitive compound was 11,14,17-eicosatrienoic acid (−5.867 kcal/mol) (Table 4).

Sucrose also showed the highest binding score of −10.002 kcal/mol to 14-α demethylase, whereas the minimum binding score of −9.101 kcal/mol was obtained with 3,7,11,15-tetramethyl-2-hexadecen-1-ol (Table 5).

The strongest binding compound from *L. nudicaulis* to 17β-hydroxysteroid dehydrogenase was the gulonic acid Ɣ-lactone (−7.811 kcal/

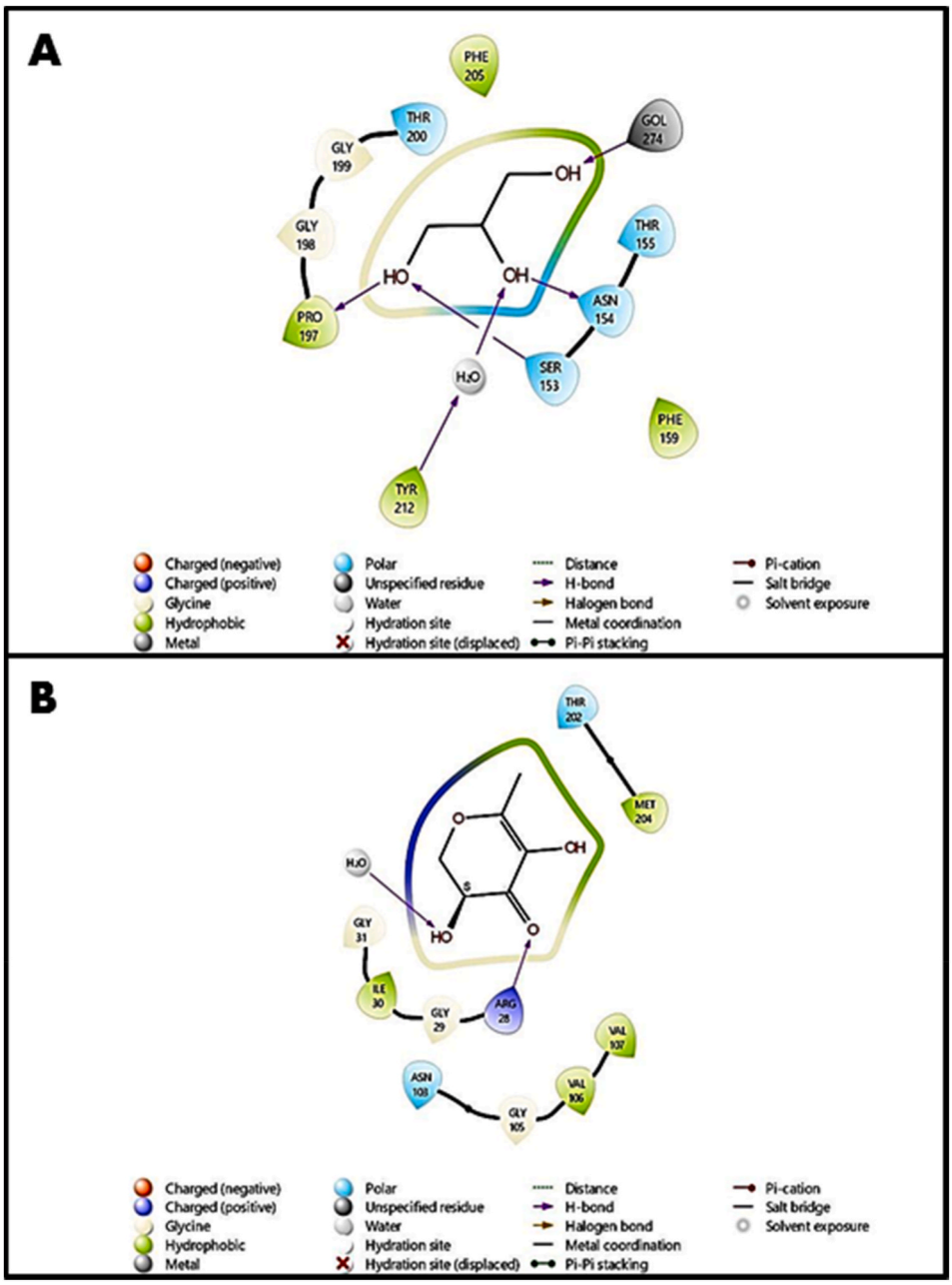


Fig. 3. Representative examples for molecular docking of *L. mucronata*, A: reference ligand, B: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl to 17β-hydroxysteroid dehydrogenase (pdb: 3IS3).

mol), which showed higher affinity compared to the reference ligand glycerol (−6.176 kcal/mol) (Table 6). On another hand, the lowest affinity to this enzyme was obtained with 2-myristynoyl-glycinamide (−3.316 kcal/mol).

Towards endochitinase, glutaric acid, di(3,4-difluorobenzyl) ester possessed the strongest affinitive bond of −8.446 kcal/mol, compared to −8.238 kcal/mol obtained by the reference ligand (Table 7). The weakest binding score was obtained with 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl (−5.584 kcal/mol).

Glutaric acid, di(3,4-difluorobenzyl) ester also exhibited the highest affinity (−9.582 kcal/mol) to 14-α demethylase, whereas the weakest affinity was obtained with oleic acid (−7.843 kcal/mol) (Table 8).

This molecular docking showed the binding ability of several compounds from both extracts to the three selected enzymes. Docking and scoring algorithms could successfully predict the protein-bound conformation, virtual screening of large data sets and sometimes to try and estimate molecule potency and help inspire further antifungal synthetic plans (Schaduangrat et al., 2020). Noncovalent interactions and chemical bonds are the primary means by which chemicals bind to recipients (Grabowski, 2020). Hydrogen bonding and hydrophobic interactions are the main determinants when assessing the binding affinity of organic compounds and their bioactive efficacy (Chen et al., 2016; Abelian et al., 2021). Indeed, hydrogen bonding plays an important role in protein-drug interactions and often occurs between protein-ligand intricacies (Grabowski, 2020). Moreover, lipophilic atoms of phytochemicals form hydrophobic interactions with nonpolar atoms of amino acid residues from the receptors active site. These interactions are good enough for the particle in question to induce a much higher binding energy than the latter; these phytochemicals also show that they are a good receptor blocker (Chen et al., 2016; Abelian et al., 2021; Faloye et al., 2023). Thus, from our results, the phytochemicals probably interacted with the amino acids of the selected proteins *via* hydrogen

Table 4
Molecular docking of phytochemical constituents identified in ethanomethanolic extract of *L. mucronata* aerial parts towards endochitinase (pdb: 4TXE).

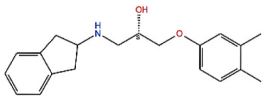
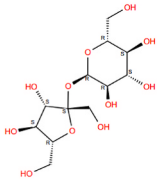
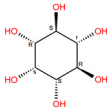
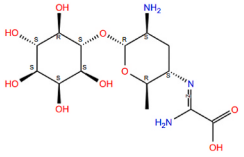
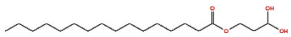
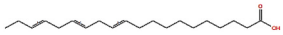
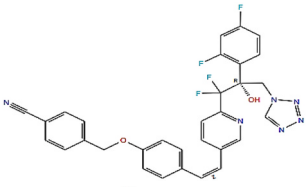
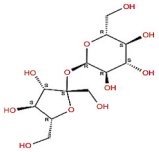
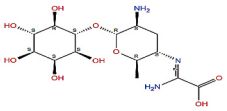
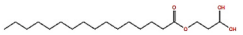
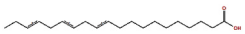
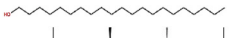
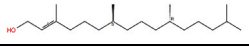
No.	Compound name	Compound 2D structure	Binding score (Kcal/mol)
1	Reference ligand (38F)		−8.23
2	Sucrose		−7.979
3	l-Inositol		−7.87
4	D-chiro-Inositol, 3-O-(2-amino-4-((carboxyiminomethyl)amino)-2,3,4,6-tetradeoxy-.α.-D-arabino-hexopyranosyl)-		−7.267
5	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester		−6.24
6	11,14,17-Eicosatrienoic acid		−5.867

Table 5
Molecular docking of phytochemical constituents identified in ethanomethanolic extract of *L. mucronata* aerial parts towards sterol 14-alpha demethylase (CYP51B) (pdb: 5FRB).

No.	Compound name	Compound 2D structure	Binding score (Kcal/mol)
1	Reference ligand (VT2)		−10.788
2	Sucrose		−10.002
3	D-chiro-Inositol, 3-O-(2-amino-4-((carboxyiminomethyl)amino)-2,3,4,6-tetradeoxy-.alpha.-D-arabino-hexopyranosyl)-		−9.782
4	Hexadecanoic acid 2-hydroxy-1-(hydroxymethyl) ethyl ester		−9.752
5	11,14,17-Eicosatrienoic acid		−9.655
6	1-Heneicosanol		−9.11
7	3,7,11,15-Tetramethyl-2-hexadecen-1-OL		−9.101

bonds, arene-arene hydrophobic, or arene-cation (Nagah et al., 2021; Nazir et al., 2023). Consequently, the inhibitory effect of these extracts is attributed to their high content of oxygenated monoterpenes, aromatic compounds, and oxygenated sesquiterpenes (Nagah et al., 2021). To well understand the molecular docking data, there is need to use artificial intelligence and deep learning that will explain the docking mechanisms of phytochemicals to amino acids. Thus, novel drugs will be discovered and designed based on ligand (Piroozmand et al., 2020).

3.4. Antifungal activity of Launaea spp.

The results of antifungal activity of *L. mucronata* and *L. nudicaulis* extracts are presented in Fig. 4.

The extract of *L. mucronata* totally inhibited *M. phaseolina* growth at 0.0001 mg/L, while the effect decreased with the increase of extract concentration. Indeed, 0.001 mg/L extract inhibited fungal growth by 87.04%, whereas 0.01 mg/L had no inhibitory effect (Fig. 4, A-B). Against *F. solani,* 0.0001 mg/L extract caused 92.4% inhibition of fungal

Table 6
Molecular docking of phytochemical constituents identified in ethanomethanolic extract of *L. nudicaulis* aerial parts towards 17β-hydroxysteroid dehydrogenase (pdb: 3IS3).

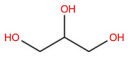
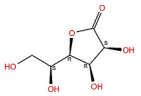
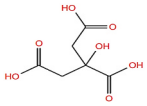
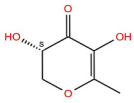
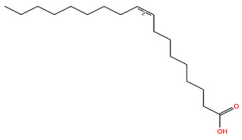
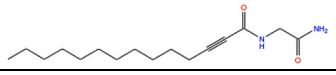
No.	Compound name	Compound 2D structure	Binding score (Kcal/mol)
1	Reference ligand (Glycerol)		−6.176
2	Gulonic acid Y-lactone		−7.811
3	Citric acid		−7.581
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl		−4.584
5	Oleic Acid		−3.975
6	2-Myristynoyl-glycinamide		−3.316

Table 7
Molecular docking of phytochemical constituents identified in ethanomethanolic extract of *L. nudicaulis* aerial parts towards Endochitinase (pdb: 4TXE).

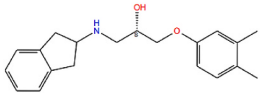
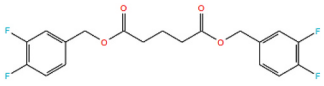
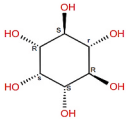
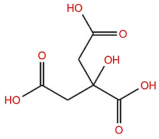
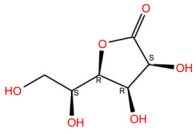
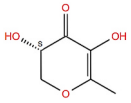
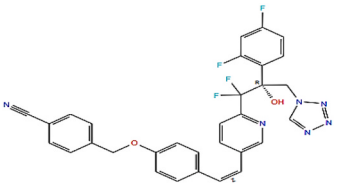
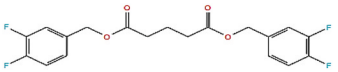
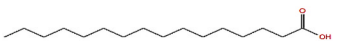
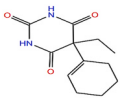
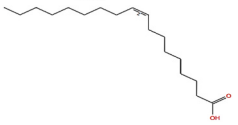
No.	Compound name	Compound 2D structure	Binding score (Kcal/mol)
1	Reference ligand (38F)		−8.238
2	Glutaric acid, di (3,4-difluorobenzyl) ester		- 8.446
3	l-Inositol		−7.87
4	Citric acid		−6.707
5	Gulonic acid gamma-lactone		−5.687
6	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl		−5.584

Table 8
Molecular docking of phytochemical constituents identified in ethanomethanolic extract of *L. nudicaulis* aerial parts towards sterol 14-alpha demethylase (CYP51B) (pdb: 5FRB).

No.	Compound name	Compound 2D structure	Binding score (Kcal/mol)
1	Reference ligand (VT2)		−10.788
2	Glutaric acid, di (3,4-difluorobenzyl) ester		−9.582
3	1-Heptacosanol		- 8.648
4	n-Hexadecanoic acid		- 8.162
5	Cyclobarbital		- 7.904
6	Oleic Acid		- 7.843

growth, while higher concentration of *L. mucronata* extract showed less inhibition percentage, with 74.8 and 66.3% inhibition obtained by 0.001 and 0.01 mg/L extract, respectively (Fig. 4, E-F).

On another hand, the ethanomethanolic extract of *L. nudicaulis* completely inhibited *M. phaseolina* at the highest concentration of 0.01 mg/L (Fig. 4), while 75.9 and 40.9% inhibition were obtained at 0.001 and 0.0001 mg/L, respectively (Fig. 4C and D). In contrary, the extract exhibited the highest inhibitory effect against *F. solani* at the smallest concentration (94.6% inhibition at 0.0001 mg/L), while increasing concentration caused a decrease of inhibitory effect (86.7 and 77.6% at 0.001 and 0.01 mg/L, respectively) (Fig. 4, G-H).

A-B: effect of *L. mucronata* extract and fractions on *M. phaseolina;* C-D: effect of *L. nudicaulis* extract and fractions on *M. phaseolina;* E-F: effect of *L. mucronata* extract and fractions on *F. solani;* G-H: effect of *L. nudicaulis* extract and fractions on *F. solani* with negative (Co-; DMSO) and positive (Co+; Azoxystrobin) controls.

The findings showed that both plants significantly inhibited fungal growth. This is in accordance with other study showing that *Launaea* spp. have antifungal activity. Abouzied et al. (2021) found that the leaf extract of *L. mucronata* showed better antifungal activity against *Candida albicans* than ketoconazole, reflecting the extract's potential as an antifungal. The methanolic extract of *L. nudicaulis* exhibited an antifungal activity on Aspergillus species, inhibiting fungal growth by half at the concentration of 0.838 mg/mL (Rashid et al., 2000). In contrary, chloroform and methanol extracts of *L. nudicaulis* showed no activity against *Aspergillus niger* and *Candida albicans* (El-Sharkawy et al., 2017).

In our study, *M. phaseolina* growth was completely inhibited by both *Launaea* spp. extracts, showing more sensitivity than *F. solani*. The extract of *L. mucronata* was a more potent antifungal than *L. nudicaulis*, since a smaller concentration (0.0001 mg/L) was sufficient to totally inhibit *M. phaseolina* growth. On another hand, *F. solani* was more sensitive to both extracts at 0.0001 mg/L compared to higher concentrations, which could be due to a better stability of extract compounds at lower concentrations (Abd-El-Fattah et al., 1990), making them more effective at inhibiting fungal growth.

The antimicrobial features of medicinal plants are increasingly

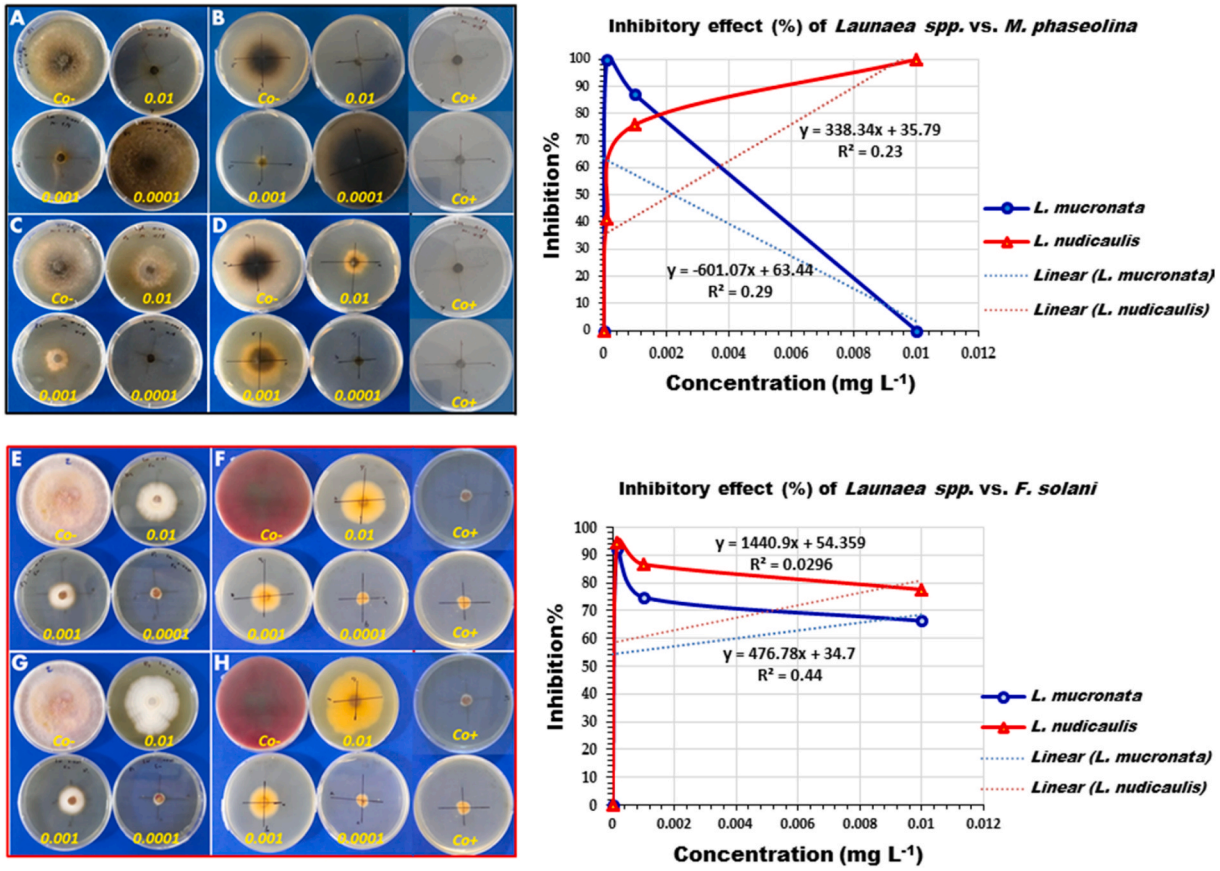


Fig. 4. The inhibitory effect of *L. mucronata* and *L. nudicaulis* ethanomethanolic extracts and fractions against *M. phaseolina* and *F. solani*.

recorded from various areas of the world. The World Health Organization assesses that the plant extract or its active ingredients are exploited as traditional medicine in folk therapies by 80% of the world population (Shaik et al., 1994). Many authors revealed that plants possess biochemicals pathways to biosynthesize thousands active compounds, those compounds possess therapeutic potential against many microorganisms. Previous study showed that two flavonoids isolated from *L. resedifolia* exhibited an antifungal activity against *Candida albicans* (Moussaoui et al., 2010). Indeed, several classes of compounds possess antifungal potential, including terpenoids, saponins, phenolic compounds (flavonoids and coumarins), alkaloids, proteins, and peptides (Aqil et al., 2010). On another hand, Bhalodia and Shukla (2011) found that *Cassia fistula* hydroalcoholic extracts have antifungal activity against some fungi species. This activity could be due to the presence of active compounds like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. These compounds possess bioactivity with inhibitory index. Thus, the antifungal activity of our extracts may be due to the presence of terpenes and terpenoids, especially lupeol, in *L. mucronata* extract and fatty acids and β-sitosterol in *L. nudicaulis* extract. Indeed, these compounds are known as potent antifungals (Aqil et al., 2010). In addition, other factors may influence the antifungal potential of plant extract such as the intrinsic biological activity of the compounds, their degree of diffusion in the study growth media (Parekh and Chanda, 2006), and even the selected susceptibility tests (Berkow et al., 2020).

4. Conclusion

GC/MS-based phytochemical analysis of the ethanomethanolic extracts shows the presence of several bioactive compounds in *Launaea mucronata* (Forssk.) Muschl. and *Launaea nudicaulis* (L.) Hook.fil. wildly growing types. Antifungal activity of the ethanomethanolic extracts was detected against plant pathogens especially at low doses. Moreover, molecular docking analysis of the major phytochemicals present in the two *Launaea* spp. with three nominated fungal proteins 17β-hydroxysteroid dehydrogenase, endochitinase, and 14-α-demethylase confirmed the antifungal activity of some selected bioactive compounds against the two targeted fungi *F. solani* and *M. phaseolina*. Consequently, the best active extracts can be subjected to antimicrobial therapeutic isolation and further fungicidal evaluation. Launaea genus could be a raw material for many studies such as therapeutic, taxonomical, drug modelling, and antifungal agent.

Credit author statement

AOA: designed the experiment, performed the measurement and analysis and drafted the manuscript, performed the experiment. MSA: designed the experiment, performed the measurement and analysis and drafted the manuscript. RMT: designed the experiment, performed the measurement and analysis and drafted the manuscript, finalized the manuscript. All authors read and approved the final manuscript. MSA: performed the experiment. RMT: performed the experiment. AOA: coordinated the study and revised the manuscript. AFA: coordinated the study and revised the manuscript. SC: coordinated the study and revised the manuscript. SC: finalized the manuscript. All authors read and approved the final manuscript. AFA: finalized the manuscript. All authors read and approved the final manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Abd-El-Fattah, H., Zaghloul, A.M., Halim, A.F., Waight, E.S., 1990. Steroid and triterpenoid constituents of Launaea resedifolia (L.) Kuntze. Egypt. J. Pharmaceut. Sci. 31 (1–4), 81–91.

Abelian, A., Dybek, M., Wallach, J., Gaye, B., Adejare, A., 2021. Chapter 6—pharmaceutical chemistry. In: Adejare, A.B. (Ed.), Remington: the Science and Practice of Pharmacy. Academic Press, Cambridge, MA, USA, pp. 105–128.

Abou-Zeid, A.M., Altalhi, A.D., Abd El-Fattah, R.I., 2008. Fungal control of pathogenic fungi isolated from some wild plants in taif governorate, Saudi arabia. Malays. J. Microbiol. 4, 30–39. https://doi.org/10.21161/mjm.01908.

Abouzied, A.S., Break, M.K.B., Younes, K., Ads, E.N., Syed, R.U., Alafnan, D., Alsulami, G., Hussein, W.A., 2021. *In vitro* antimicrobial, anticancer, and apoptosis-inducing effects of the methanolic extract of *Launaea mucronata*. Not. Bot. Horti Agrobot. Cluj-Napoca 49, 12556. https://doi.org/10.15835/nbha49412556.

Ahmed, Z., Ali, D., Malik, A., 2006. Structure determination of ursene-type triterpenes by NMR techniques. Magn. Reson. Chem. 44, 717–719. https://doi.org/10.1002/mrc.1803.

Al-Mahrezi, J.A., Al-Sabahi, J.N., Akhtar, M.S., Selim, D., Weli, A.M., 2011. Essential oil composition and antimicrobial screening of *Launaea nudicaulis* grown in Oman. Int. J. Pharma Sci. Res. 2, 3166–3169.

Alami, M.M., Ouyang, Z., Zhang, Y., Shu, S., Yang, G., Mei, Z., Wang, X., 2022. The current developments in medicinal plant genomics enabled the diversification of secondary metabolites' biosynthesis. Int. J. Mol. Sci. 23 (24), 15932.

Alfalahi, A.O., Hussein, Z.T., Khalofah, A., Sadder, M.T., Qasem, J.R., Al-Khayri, J.M., Jain, S.M., Almehemdi, A.F., 2022. Epigenetic variation as a new plant breeding tool: a review. J. King Saud Univ. Sci. 34, 102302 https://doi.org/10.1016/j.jksus.2022.102302, 2022.

Aqil, F., Zahin, M., Ahmad, I., Owais, M., Khan, M.S.A., Bansal, S.S., Farooq, S., 2010. Antifungal activity of medicinal plant extracts and phytocompounds: a review. In: Ahmad, I., Owais, Mohammad, Shahid, M., Aqil, F. (Eds.), Combating Fungal Infections. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 449–484. https://doi.org/10.1007/978-3-642-12173-9_19.

Benmeddour, T., Laouer, H., Akkal, S., Flamini, G., 2015. Chemical composition and antibacterial activity of essential oil of *Launaea lanifera* Pau grown in Algerian arid steppes. Asian Pac. J. Trop. Biomed. 5, 960–964. https://doi.org/10.1016/j.apjtb.2015.07.025.

Berkow, E.L., Lockhart, S.R., Ostrosky-Zeichner, L., 2020. Antifungal susceptibility testing: current approaches. Clin. Microbiol. Rev. 33 (3), e00069-19.

Bhalodia, N.R., Shukla, V.J., 2011. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: an ethnomedicinal plant. "J. Adv. Pharm. Technol. Research"" (JAPTR)" 2 (2), 104.

Bremer Christensen, C., Soelberg, J., Stensvold, C.R., Jager, A.K., 2015. Activity of medicinal plants from Ghana against the parasitic gut protist Blastocystis. J. Ethnopharmacol. 174, 569–575. https://doi.org/10.1016/j.jep.2015.03.006.

Chehri, K., Salleh, B., Zakaria, L., 2015. Morphological and phylogenetic analysis of *Fusarium solani* species complex in Malaysia. Microb. Ecol. 69, 457–471. https://doi.org/10.1007/s00248-014-0494-2.

Chen, D., Oezguen, N., Urvil, P., Ferguson, C., Dann, S.M., Savidge, T.C., 2016. Regulation of protein-ligand binding affinity by hydrogen bond pairing. Sci. Adv. 2 (3), e1501240 https://doi.org/10.1126/sciadv.1501240.

Cheriti, A., Belboukhari, M., Belboukhari, N., Djeradi, H., 2012. Phytochemical and biological studies on Launaea cass. Genus (Asteraceae) from Algerian sahara. Curr. Top. Phytochemistry 11, 67–80.

Dawood, K.F., Alfalahi, A.O., Neama, S.I., 2020. Biochemical Characterization of *Ferula rutbaensis* medicinal plant in Iraqi western desert. Periodico Tche Quimica 36 (17), 831–844.

de Boer, H.J., Kool, A., Broberg, A., Mziray, W.R., Hedberg, I., Levenfors, J.J., 2005. Anti-fungal and anti-bacterial activity of some herbal remedies from Tanzania. J. Ethnopharmacol. 96, 461–469. https://doi.org/10.1016/j.jep.2004.09.035.

El-Newary, S.A., Afifi, S.M., Aly, M.S., Ahmed, R.F., El Gendy, A.E.-N.G., Abd-ElGawad, A.M., Farag, M.A., Elgamal, A.M., Elshamy, A.I., 2021. Chemical profile of *Launaea nudicaulis* ethanolic extract and its antidiabetic effect in streptozotocin-induced rats. Molecules 26, 1000. https://doi.org/10.3390/molecules26041000.

El-Sharkawy, E.R., Mahmoud, K., 2016. Cytotoxity of two new coumarin derivatives isolated from *Launaea mucronata*. Nat. Prod. Res. 30, 394–398. https://doi.org/10.1080/14786419.2015.1017492.

El-Sharkawy, E.R., Ed-Dra, A., Abdallah, E.M., 2017. Phytochemical, antimicrobial and antioxidant properties of *Launaea nudicaulis* and *Farsetia hamiltonii*. J. Biol. Control 31, 102–109. https://doi.org/10.18311/jbc/2017/15910.

Elshamy, A.I., Abd-ElGawad, A.M., El-Amier, Y.A., El Gendy, A.E.G., Al-Rowaily, S.L., 2019. Interspecific variation, antioxidant and allelopathic activity of the essential oil from three *Launaea* species growing naturally in heterogeneous habitats in Egypt. Flavour Fragrance J. 34, 316–328. https://doi.org/10.1002/ffj.3512.

Elsharkawy, E.R., 2017. Isolation of phytoconstituents and evaluation of anticancer and Antioxidant potential of *Launaea mucronata* (Forssk.). Muschl. subsp. Pak. J. Pharm. Sci. 30, 399–405.

Faloye, K.O., Tripathi, M.K., Adesida, S.A., Oguntimehin, S.A., Oyetunde, Y.M., Adewole, A.H., Dosumu, O.D., 2023. Antimalarial potential, LC-MS secondary metabolite profiling and computational studies of Zingiber officinale. J. Biomol. Struct. Dyn. 1–16.

Geddes-McAlister, J., Shapiro, R.S., 2019. New pathogens, new tricks: emerging, drug-resistant fungal pathogens and future prospects for antifungal therapeutics. Ann. NY. Aca. Sci. 1435 (1), 57–78.

Gow, N.A., Johnson, C., Berman, J., Coste, A.T., Cuomo, C.A., Perlin, D.S., Edgar, K., 2022. The importance of antimicrobial resistance in medical mycology. Nat. Commun. 13 (1), 5352.

Grabowski, S.J., 2020. Intramolecular hydrogen bond energy and its decomposition—O–H•••O interactions. Crystals 11 (1), 5. https://doi.org/10.3390/cryst11010005.

Hawkins, P.C.D., Skillman, A.G., Nicholls, A., 2007. Comparison of shape-matching and docking as virtual screening tools. J. Med. Chem. 50, 74–82. https://doi.org/10.1021/jm0603365.

Khan, R.A., Khan, M.R., Sahreen, S., Bokhari, J., Rashid, U., Jan, S., 2011. Phytotoxic characterization of various fractions of *Launaea procumbens*. Afr. J. Biotechnol. 10, 5377–5380. https://doi.org/10.5897/AJB10.2008.

Khan, R.A., Khan, M.R., Sahreen, S., Ahmed, M., 2012a. Assessment of flavonoids contents and *in vitro* antioxidant activity of *Launaea procumbens*. Chem. Cent. J. 6, 1–11. https://doi.org/10.1186/1752-153X-6-43.

Khan, R.A., Khan, M.R., Sahreen, S., Shah, N.A., Khan, A.M., Bokhari, J., Rashid, U., Ahmad, B., Shabbir, M., Saeed, N., Jan, S., Afsar, T., 2012b. Protective effect of *Launaea procumbens* against KBrO3 induced nephrotoxicity in rats. African J. Pharm. Pharmacol. 6, 317–321. https://doi.org/10.5897/AJPP11.474.

Makasana, A., Ranpariya, V., Desai, D., Mendpara, J., Parekh, V., 2014. Evaluation for the anti-urolithiatic activity of *Launaea procumbens* against ethylene glycol-induced renal calculi in rats. Toxicol Rep 1, 46–52. https://doi.org/10.1016/j.toxrep.2014.03.006.

Mandaville, J.P., 2019. Bedouin Ethnobotany: Plant Concepts and Uses in a Desert Pastoral World. University of Arizona Press.

Mishra, A., Gupta, P., Lal, R.K., Dhawan, S.S., 2021. Assessing and integrating the transcriptome analysis with plant development, trichomes, and secondary metabolites yield potential in Mentha arvensis L. Plant Physiol. Biochem. 162, 517–530.

Moussaoui, F., Zellagui, A., Segueni, N., Touil, A., Rhouati, S., 2010. Flavonoid constituents from Algerian *Launaea resedifolia* (O.K.) and their antimicrobial activity. Record Nat. Prod. 4, 91–95.

Nagah, N., Mostafa, I., Dora, G., El-Sayed, Z., Ateya, A.M., 2021. Essential oil composition, cytotoxicity against hepatocellular carcinoma, and macro and micro-morphological fingerprint of *Laurus nobilis* cultivated in Egypt. Egypt. J. Bot., Le 61, 521–540. https://doi.org/10.21608/ejbo.2021.56342.1603.

Nazir, S., Abdel-Ghani, N.T., Atia, M.A.M., El-Sherif, A.A., 2023. Exploring the effectiveness of *Lepidium sativum* for inhibiting human hepatocellular carcinoma cell lines: *In vitro* analysis, molecular docking, and pathway enrichment analysis. Egypt. J. Bot. 63, 251–264. https://doi.org/10.21608/ejbo.2022.118677.1891.

Neama, S.E., Almehemdi, A.F., 2018. Some aspects of breeding of medicinal and aromatic plants. Iraqi. J. Des. Stud. 8 (2), 110–132.

Noumi, Z., Dhaou, S.O., Derbel, S., Chaieb, M., 2010. The status of Asteraceae in the arid and saharan flora of North African region: case of Tunisia. Pakistan J. Bot. 42, 1417–1422.

Pandit, M.A., Kumar, J., Gulati, S., Bhandari, N., Mehta, P., Katyal, R., Rawat, C.D., Mishra, V., Kaur, J., 2022. Major biological control strategies for plant pathogens. Pathogens 11, 1–21. https://doi.org/10.3390/pathogens11020273.

Parekh, J., Chanda, S., 2006. *In-vitro* antimicrobial activities of extracts of *Launaea procumbens* roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). Afr. J. Biomed. Res. 9, 89–93. https://doi.org/10.4314/ajbr.v9i2.48780.

Pinto, C.M.F., Maffia, L.A., Casali, V.W.D., Cardoso, A.A., 1998. In vitro effect of plant leaf extracts on mycelial growth and sclerotial germination of *Sclerotium cepivorum*. J. Phytopathol. 146, 421–425. https://doi.org/10.1111/j.1439-0434.1998.tb04775.x.

Piroozmand, F., Mohammadipanah, F., Sajedi, H., 2020. Spectrum of deep learning algorithms in drug discovery. Chem. Biol. Drug Des. 96 (3), 886–901.

Rashid, S., Ashraf, M., Bibi, S., Anjum, R., 2000. Antibacterial and antifungal activities of *Launaea nudicaulis* (roxb.), and *Launaea resedifolia* (linn.). Pakistan J. Biol. Sci. 3, 630–632.

Riaz, N., Parveen, S., Saleem, M., Ali, M.S., Malik, A., Ashraf, M., Afzal, I., Jabbar, A., 2012. Lipoxygenase inhibitory sphingolipids from *Launaea nudicaulis*. J. Asian Nat. Prod. Res. 14, 545–554. https://doi.org/10.1080/10286020.2012.680440.

Rohloff, J., 2015. Analysis of phenolic and cyclic compounds in plants using derivatization techniques in combination with GC-MS-based metabolite profiling. Molecules 20 (2), 3431–3462. https://doi.org/10.3390/molecules20023431.

Saleem, M., Parveen, S., Riaz, N., Tahir, M.N., Ashraf, M., Afzal, I., Ali, M.S., Malik, A., Jabbar, A., 2012. New bioactive natural products from *Launaea nudicaulis*. Phytochem. Lett. 5, 793–799. https://doi.org/10.1016/j.phytol.2012.09.004.

Sambrook, J., Russell, D.W., 2001. Molecular Cloning: A Laboratory Manual, third ed. Cold Spring Harbor Laboratory Press, New York.

Schaduangrat, N., Lampa, S., Simeon, S., Gleeson, M.P., Spjuth, O., Nantasenamat, C., 2020. Towards reproducible computational drug discovery. J. Cheminf. 12 (1), 30. https://doi.org/10.1186/s13321-020-0408-x.

Shaik, D., Malika, F.A., Rafi, S.M., Naqui, B., 1994. Studies of antibacterial activity of ethanolic extract from *Nericum indicum* and *Hibiscus rosasinensis*. J. Islamic Acad. Sci. 7, 167–168.

Veverka, K., Palicová, J., Křížková, I., 2008. The incidence and spreading of *Macrophomina phaseolina* (Tassi) Goidanovich on sunflower in the Czech Republic. Plant Protect. Sci. 44, 127–137. https://doi.org/10.17221/31/2008-pps.

Vogelstein, B., Gillespie, D., 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76, 615–619. https://doi.org/10.1073/pnas.76.2.615.

Wang, Z., Yang, Z., Li, F., 2019. Updates on molecular mechanisms in the development of branched trichome in Arabidopsis and nonbranched in Cotton. Plant Biotechnol. J. 17, 1706–1722. https://doi.org/10.1111/pbi.13167.